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MODE OF ACTION OF CHICKPEA ANTAGONISTIC BACTERIA ON *RHIZOCTONIA BATATICOLA* UNDER *IN VITRO*

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ABSTRACT

The in vitro volatile experimental results revealed that, out of three endophytic and one rhizospheric chickpea bacteria tested against Rhizoctonia bataticola, causal agent of dry root rot, volatile metabolites produced by antagonistic bacteria (CREB-16) showed highest inhibition to an extent of 77.77%, followed by CRB-6 (72.22%) and CREB-13 (71.11%). CRB-13 showed least inhibiton (55.55%) over control. Non volatile metabolites has no inhibitory action upon the pathogen for all four bacteria.

KEYWORDS: Chickpea, Rhizoctonia Bataticola, CREB-16, CREB-13, CREB-6, CRB-13, Volatiles, Non Volatiles

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Original Article

INTRODUCTION

Chickpea is the third important legume grown around the world, which has world wide acceptance as a major source of protein and essential amino acids for human as well as for animal consumption. India is the leading producer of chickpea contributing to about 70 per cent of the world's chickpea production (Anonymous, 2014-2015). Chickpea suffers from several fungal diseases, out of these dry root rot (*Rhizoctonia bataticola* (Taub.) Butler) cause considerable yield losses in chickpea which may be as high as 50 to 71 per cent (Ahmed and Mohammad, 1986). Soil borne pathogens are difficult to control because of their wide host range and long term survival in the soil. Biological control using native microbial antagonists is considered as good alternative of management of root diseases in many crops (Cook and Baker, 1983). Taking into consideration the above facts, the present investigation has been formulated to know the mechanism of antagonism of chickpea rhizospheric and endophytic bacteria against *Rhizoctonia bataticola*.

MATERIALS AND METHODS

Isolation of Pathogen

The pathogen was isolated from dry root rot infected chickpea plants by using tissue segment method (Rangaswamy and Mahadevan, 1999).

Identification of Pathogen

The pathogen was identified based on its mycelial and sclerotial characters as described by Barnett and Barry (1972).

Isolation of Bacterial Biocontrol Agents

Rhizospheric and root endophytic bacteria were isolated and tested against *Rhizoctonia bataticola* by placing antagonist and pathogen at ends of the Petri plate under *in vitro* conditions (Morton and Stroube, 1995). The isolates which performed well in dual culture studies were tested for volatile and non volatile metabolites.

Antibiosis

The effect of volatile and non-volatile metabolites produced by the potential antagonistic bacteria on *Rhizoctonia bataticola* was studied under *in vitro* conditions.

Effect of Volatile Metabolites of Bacterial Antagonists on Radial Growth of *Rhizoctonia bataticola*

Modified version of sealed Petri plate technique described by Dennis and Webster (1971) was used to study the influence of volatile substances on the growth of *Rhizoctonia bataticola.*

In this case, the bacterial antagonist from 48 h old culture was streaked at the centre of the petri plate containing nutrient agar and the lid of this Petri plate was replaced by another bottom plate containing PDA medium inoculated with six mm actively growing culture disc of *Rhizoctonia bataticola*. The two dishes were sealed together with an adhesive tape. The lid of control plate, which had not been inoculated with antagonist served as a control. Three replications were maintained and the plates were incubated at 28 ± 2°C. The radial growth of the pathogen was recorded after every 24 h and compared with the growth of pathogen in control. Based on the observations recorded, percentage inhibition of the pathogen was calculated by the formula.

$$I = \frac{C - T}{C} X 100$$

where,

I = Per cent inhibition in growth of test pathogen

C = Radial growth (cm) in control

T = Radial growth (cm) in treatment.

Effect of Non-Volatile Metabolites of Bacterial Antagonists on Radial Growth of *Rhizoctonia Bataticola*

The effect of culture filtrate of bacterial antagonists on the growth of *Rhizoctonia bataticola* was studied as per the method given by Dennis and Webster (1971).

Bacterial antagonists will be cultured in 100 ml of nutrient broth in 250 ml Erlenmeyer flask with intermittent shaking. After two days, the culture filtrate will be passed through what man No. 42 filter paper and the filtrate will be centrifuged at 3000 rpm for 10 min and sterilized by passing it through Millipore membrane filter (0.4 µm pore size). Filtered and sterilized culture filtrate of the bacterial antagonist grown for two days on Nutrient broth was added to molten PDA and the medium was poured into Petri plates and plates were inoculated with six mm disc of *Rhizoctonia bataticola*. Control plates are without culture filtrate. Observations on the radial growth of test pathogen were recorded after seven days of incubation at 28 ± 2^0C. The radial growth of the test fungus in the treatment in comparison with that of control was recorded and the per cent inhibition was calculated.

RESULTS AND DISCUSSIONS

Identification of Pathogen

Dark brown mycelium, right angled branching and dark sclerotia resembles the pathogen as *Rhizoctonia bataticola.*

Figure 1: Pure culture of *Rhizoctonia bataticola* (Mycelial Stage)

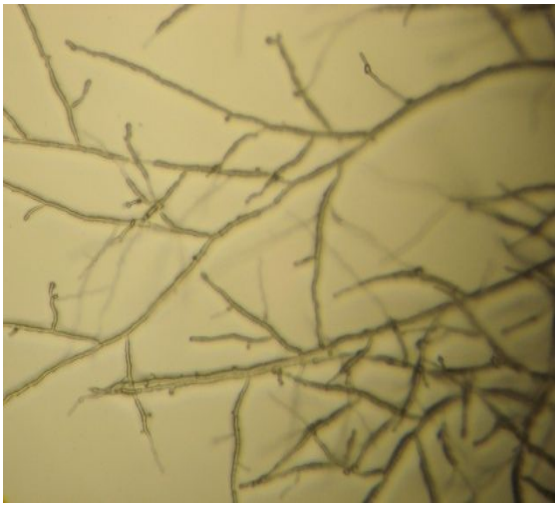

Figure 2: Photomicrograph of *Rhizoctonia Bataticola* Showing Septate and Branched Hyphae (10x) (Indicated by arrows)

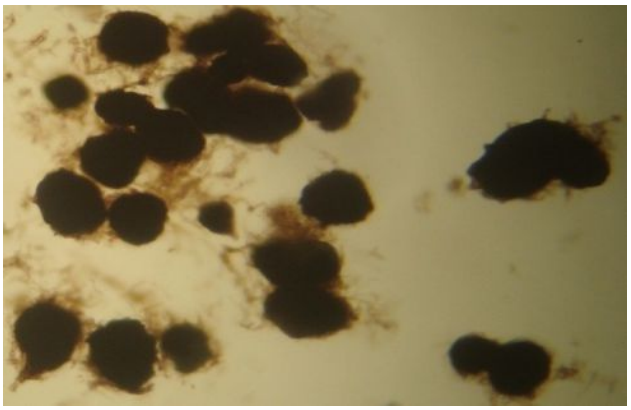

Figure 3: Photomicrograph of sclerotia of *Rhizoctonia bataticola* (10x)

Identification of Bacterial Biocontrol Agents

Twenty Rhizospheric bacteria were designated as CRB-1 to CRB-20 (Figure 4) followed by twenty root endophytic bacteria as Chickpea Root Endophytic Bacteria-1 (CREB-1) to Chickpea Root Endophytic Bacteria-20 (CREB-20) (Figure 5). Four isolates which showed highest inhibition in dual culture studies were tested for volatile and non volatile metabolites.

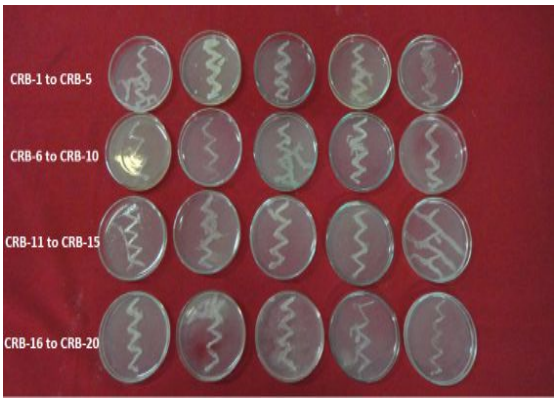

Figure 4: Pure cultures of Chickpea Rhizosphere Bacterial isolates (CRB 1 to 20)

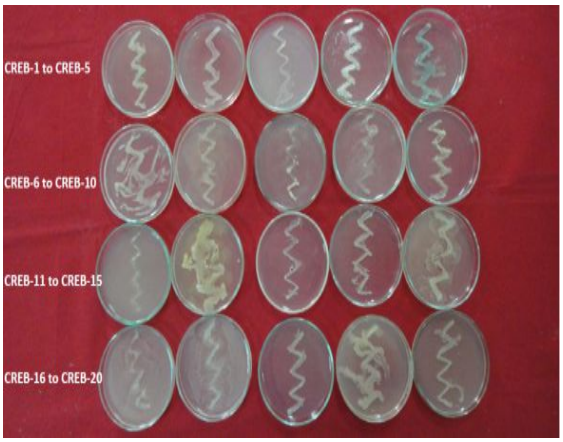

Figure 5: Pure cultures of Chickpea Root Endophytic Bacterial isolates (CREB 1 to 20)

Effect of Volatile Metabolites of Potential Antagonistic Bacteria Against Rhizoctonia Bataticola

The *in vitro* experimental results (Figure 6) revealed that volatile metabolites produced by antagonistic bacteria CREB-16 has shown maximum inhibition of the mycelial growth of *Rhizoctonia bataticola* to an extent of 77.77 per cent, followed by CRB-6 (72.22 per cent) and CREB-13 (71.11 per cent). CRB-13 showed least inhibiton (55.55 per cent) against *Rhizoctonia bataticola*. The inhibitory effect of the bacterial antagonist on *Rhizoctonia bataticola* may be due to the production of some volatile metabolites like hydrogen cyanide.

Prasanna Reddy *et al*. (2010) isolated rhizospheric *Pseudomonas fluorescens* strains from rice plants in Andhra Pradesh and Tamil nadu. Volatile metabolite from *Pseudomonas fluorescens* isolate (P.f 003) gave 78 per cent inhibition against *Rhizoctonia solani* compared to control.

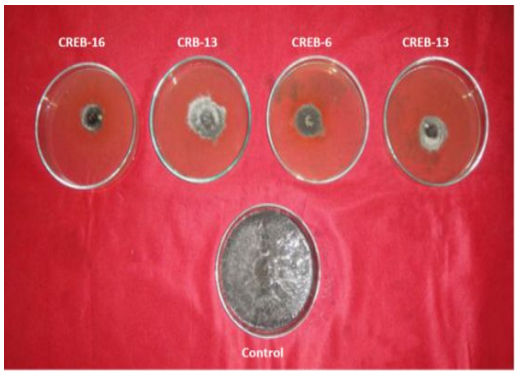

Figure 6

Table 1

S. No.	Bacterial Antagonists	Linear growth of *Rhizoctonia Bataticola* (cm)*	Per cent Inhibition
1	CREB-13	2.6	71.11 (57.48)
2	CREB-6	2.5	72.22 (58.18)
3	CREB-16	**2.0**	**77.77 (61.82)**
4	CRB-13	4.0	55.55 (48.16)
5	Control	9.0	-
	S. Em ±		0.68
	CD (0.05)		2.37

* Mean of three replications

Figure ures in parenthesis are angular transformed values

Effect of Non Volatile Compounds of Potential Antagonistic Bacteria Against Rhizoctonia Bataticola

The results of the effect of non-volatile compounds of the bacterial antagonist (Figure 7) against the test pathogen are presented below. The effect of culture filtrates of antagonistic bacteria on radial growth of *Rhizoctonia bataticola* indicated that the antagonists did not have any inhibitory action on the growth of *Rhizoctonia bataticola* over control under *in vitro*.

The results revealed that there was no reciprocal relationship between the culture filtrates of antagonistic bacteria and the radial growth of *Rhizoctonia bataticola* indicating that they did not produced any inhibitory non-volatile metabolites against *Rhizoctonia bataticola*.

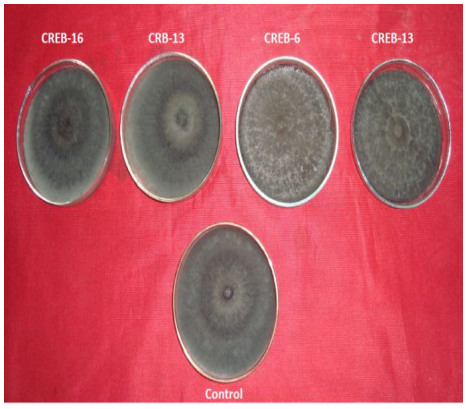

Figure 7

Table 2

S. No.	Bacterial Antagonists	Linear Growth of *Rhizoctonia Bataticola* (cm)	Per Cent Inhibition
1	CREB-13	9.0	0.00
2	CREB-6	9.0	0.00
3	CREB-16	9.0	0.00
4	CRB-13	9.0	0.00
5	Control	9.0	-

CONCLUSIONS

CREB-16 antagonistic bacteria inhibited the mycelial growth of *Rhizoctonia bataticola* to an extent of 77.77 per cent followed by CREB-6 (72.22 %) and least with the isolate CRB-13 (55.55%) in the case of volatile test. Non volatile metabolites from four bacteria were not effective against the pathogen as the pathogen completely occupied the Petri plate same as control plates.

FUTURE RESEARCH

The chemical compounds responsible for supression of pathogen radial growth in volatile metabolites test will be identified based on biochemical tests and the product will be prepared for field application.

ACKNOWLEDGEMENTS

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